

Supplementary information, Fig. S4. In this figure, HeLa cells were treated with DM- α KG (15 mM) for 6 hours to observe the protein localization under confocal microscopy, or 24 hours to assess DR6 oxidation and pyroptotic features (including cell morphology, GSDMC cleavage, and LDH release), unless specially indicated otherwise.

(a, b) DM- α KG induced DR6 colocalization with either caspase-8 (a) or GSDMC (b) as observed under confocal microscopy. Cells were transfected with different plasmids as indicated.

(c) Knocking down caspase-8 (left) or GSDMC (right) had no effect on DM- α KG-induced DR6 puncta formation. DR6-GFP was transfected into caspase-8- or GSDMC-knockdown cells.

(d) Co-IP assay indicated the interaction between DR6-caspase-8 (left) and DR6-GSDMC (right). Different plasmids were transfected into cells as indicated.

(e) The knockdown efficiency of FADD and TRADD in the cells as determined by western blotting.

(f) Knocking down TRADD had no effect on DM- α KG induced pyroptosis, including cell morphology and LDH release.

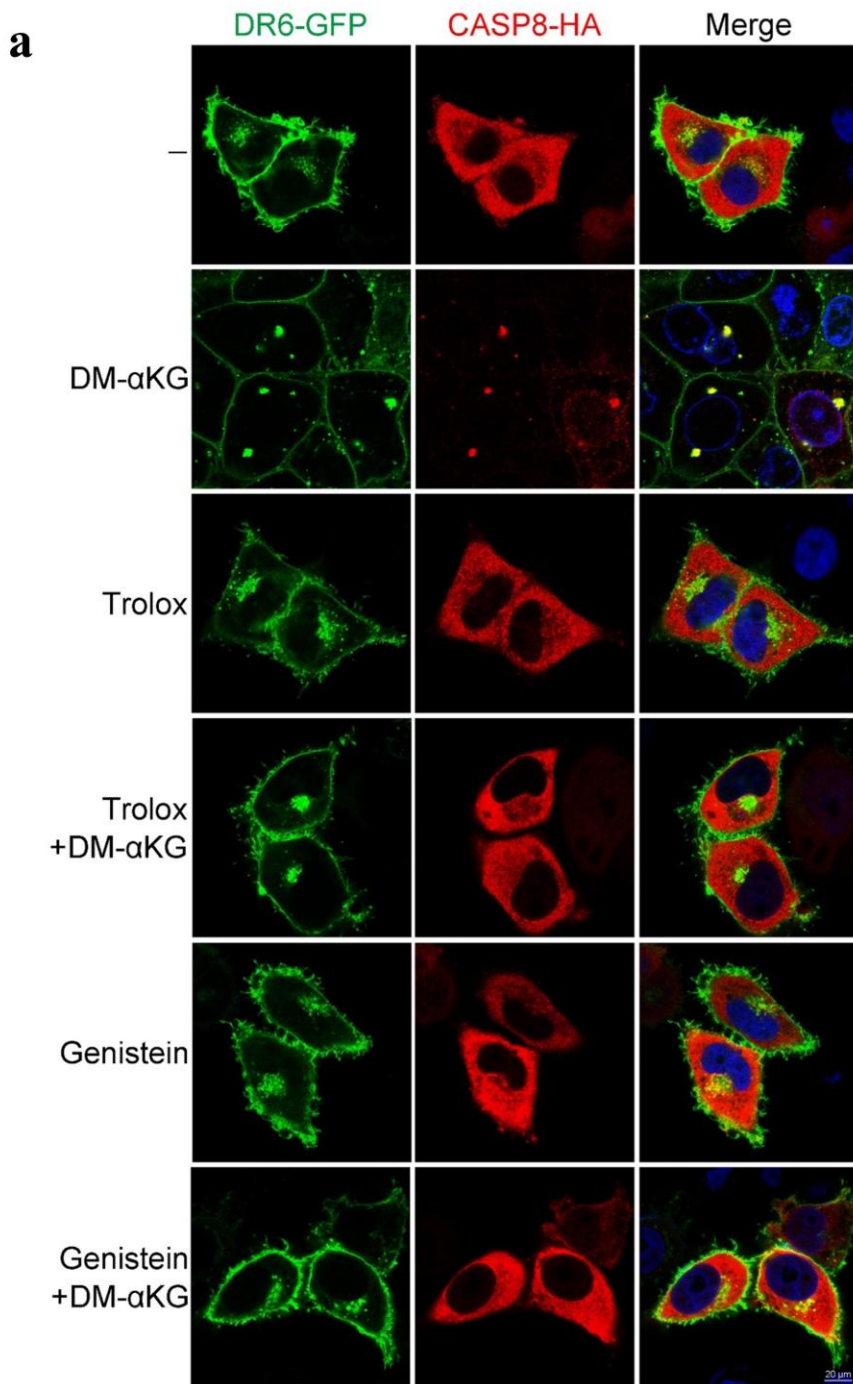
(g) Genistein impaired the DM- α KG-induced plasma membrane localization of

GSDMC-N.

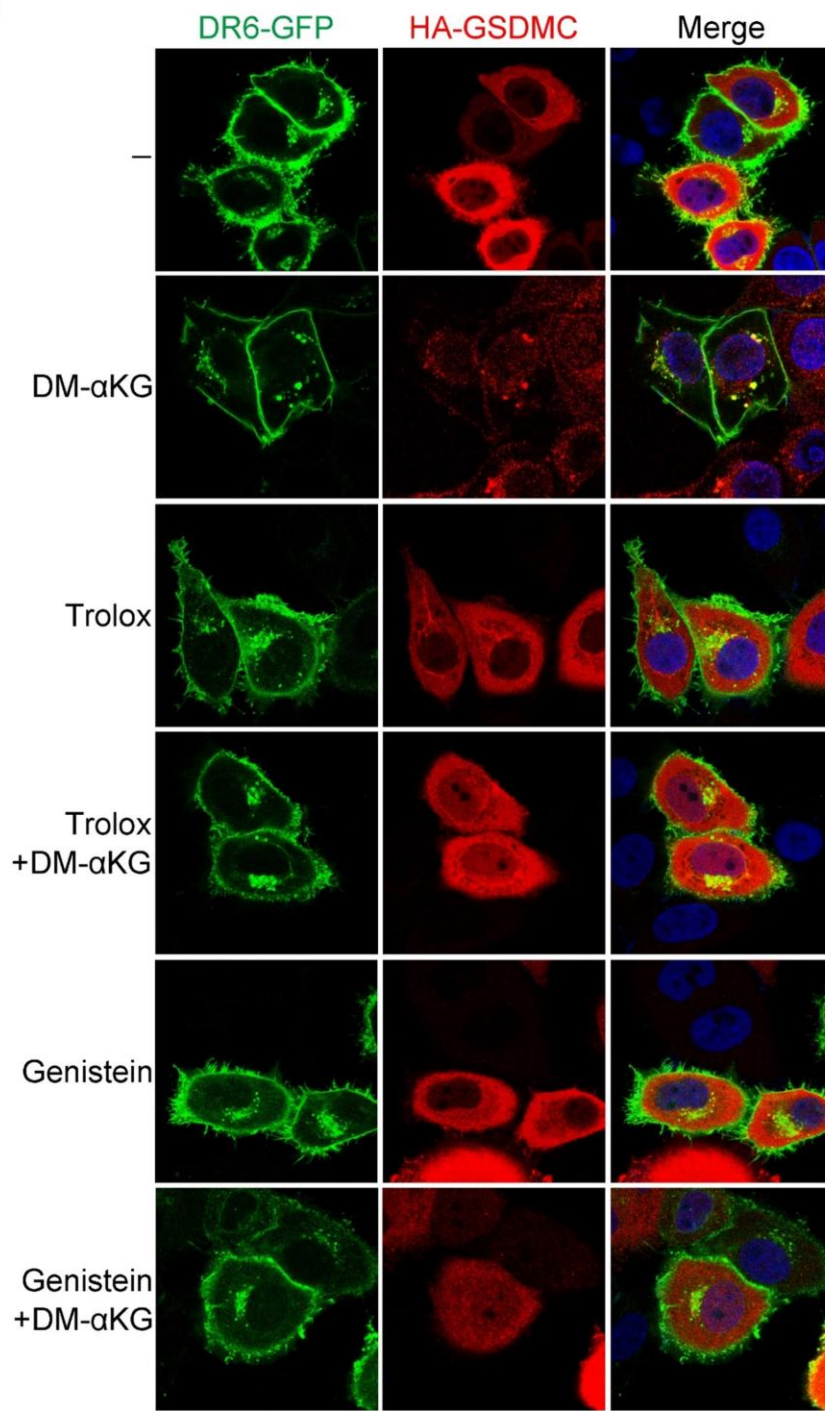
(h) TNF α /CHX but not DM- α KG induced the cleavage of Bid and caspase-3.

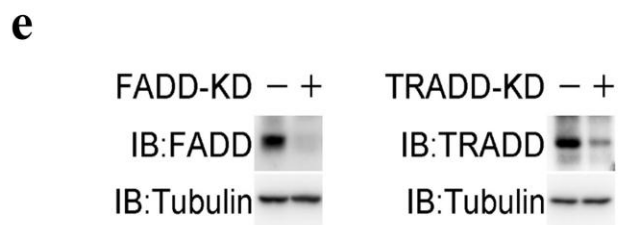
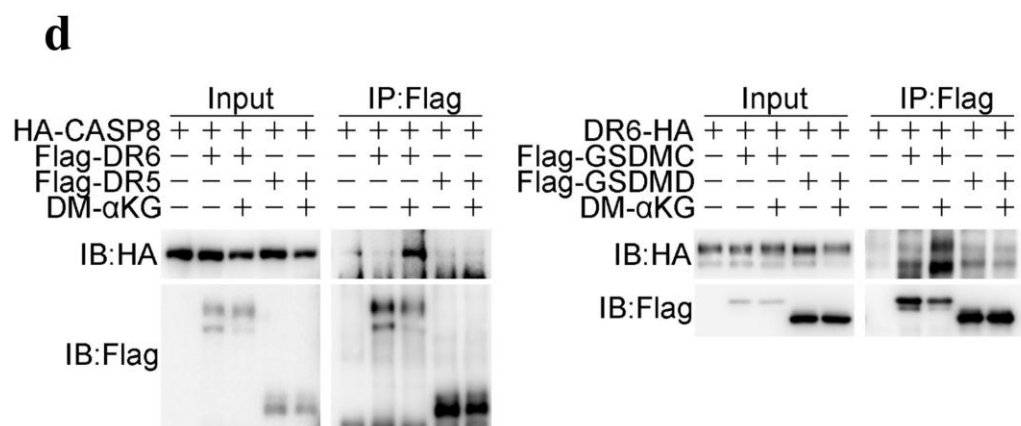
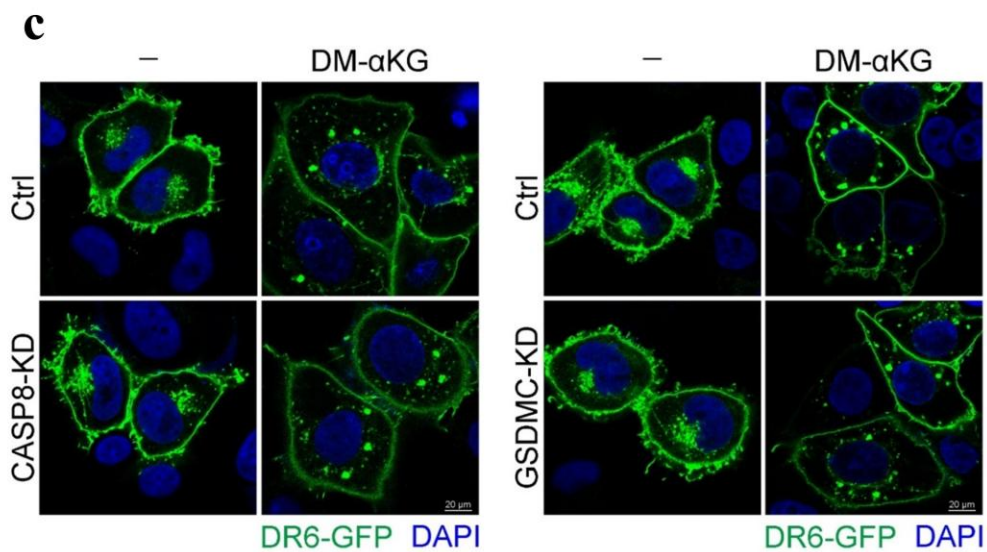
Tubulin was used to determine the amount of loading proteins. All data are presented as the mean \pm SEM of two or three independent experiments. ns: not significant. The data were analyzed two-way ANOVA followed by the Bonferroni test.

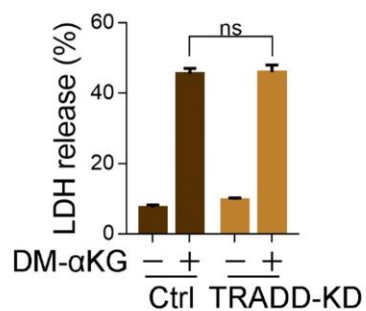
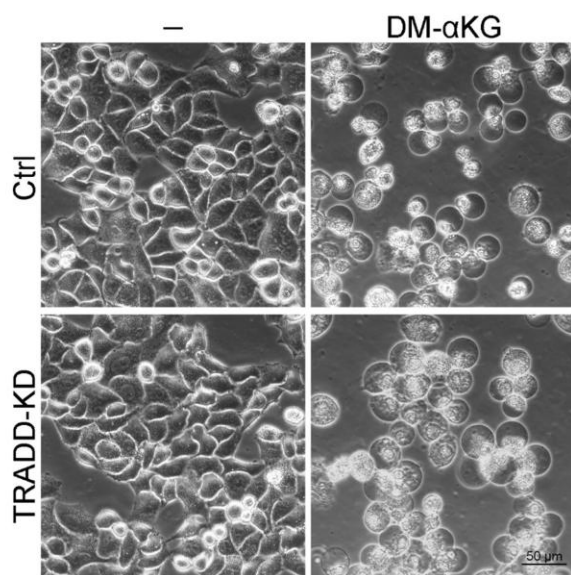
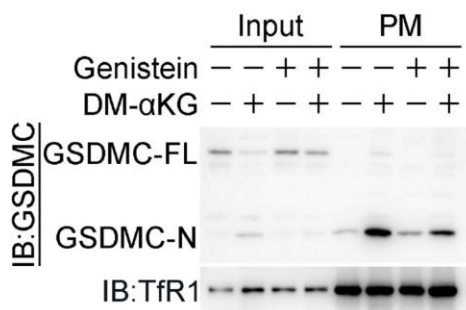
Supplementary information, Figure S4



b





f**g****h**